BIOLABO
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U.S. PROTEIN Pyrogallol Red Method

Reagent for quantitative determination of protein in human urines and cerebrospinal fluid (CSF).

I REF K2017 **R1** 4 x 40 mL **R3** 1 x 5 mL

CE

IVD

Made In France

I: corresponds to significant modifications

TECHNICAL SUPPORT AND ORDERS
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Latest revision: www.biolabo.fr

INTENDED USE

This reagent is designated for professional use in laboratory (automated method).
It allows the for quantitative determination of protein in human urines and cerebrospinal fluid (CSF).

GENERALITIES (1)

The determination of total protein in urines and in the cerebrospinal fluid (C.S.F.) is respectively used for help to diagnose of renal or central nervous system diseases. A rise in the concentration in urinary proteins is commonly seen in following cases: vigorous effort, fever or hypothermia, monoclonal gammopathies, nephropathy, diabetic nephropathy or urinary tract infection.
The determination of total protein in the CSF represents a help to the diagnosis of meningitis, encephalitis, poliomyelitis, neurosyphilis, tumours of the central nervous system or cerebral haemorrhage.

PRINCIPLE (4) (7)

Fujita's method modified by Watanabe and al. Pyrogallol red combined with sodium molybdate forms a red coloured complex which absorbs at 460 nm. In an acid medium, the fixation of this complex on the amino groups of the proteins moves the absorption peak to 600 nm. The intensity of the blue staining measured at 600 nm (578-612) is proportional to the concentration of proteins in the specimen.

REAGENTS

R1	**U.S. Protein**	Working Reagent
Sodium Molybdate	0.04	mmol/L
Methanol	10.4	%
Pyrogallol Red	0.06	mmol/L

Caution, Danger
STOT SE1: H370 - Causes damage to organs
Acute Tox.4: H302 – Harmful if swallowed,
P260: Do not breath vapors, P264: wash thoroughly after handling.
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell,
P330: Rinse mouth, P405: Store locked up.
P501: Dispose of contents/container in accordance with dangerous waste regulations
Classification due to: Methanol: 10 - < 25%. For more details, refer to Safety data sheet (MSDS)

R3	**U.S. Protein**	Standard
Bovine Albumin		approx. 100 mg/dL

Specific batch value indicated on the label of the vial.

According to 1272/2008/EC regulation, this reagent is not classified as dangerous

SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENTS PREPARATION

Ready for use.

STABILITY AND STORAGE

Stored away from light, well cap in the original vial at 18-25°C, reagent is stable when stored and used as described in the insert:

Unopened,
- Until the expiry date stated on the label of the Kit.

Once opened:
- Discard any reagent if cloudy or if reagent blank at 620 nm > 0.600.

SPECIMEN COLLECTION AND HANDLING (2)

Urines: Micturition or partial collection.

24 h Urines: Freshly collected urines, stored at 2-8°C.
No preservative requested, centrifuge 10 minutes at 3000 RPM and adjust pH at 7.0.

Stability in urines:
- Over 1 year at –20°C.

CSF: Freshly collected and centrifuged before assay.
Avoid specimen containing blood.

Stability in CSF:
- up to 72 h at 2-8°C.
- 6 months at - 20°C.
- Indefinitely at –70°C.

LIMITS (3) (5)

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Biochemistry Clinical Analyzer Kenza One, Kenza 240TX/ISE or Kenza 450TX/ISE

EXPECTED VALUES (2)

Urines (micturition)	< 14.0 mg/dL
24 h Urines	
At rest	< 80 mg/24 h
After intensive exercise	< 250 mg/24 h

CSF	mg/dL
Premature	15 - 130
Newborn	40 – 120
< 1month	20 - 80
> 1 month	15 – 40

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES

On Kenza 240TX analyser, at 620 nm, 37°C
Linearity Range: between 12 and 138 mg/dL
Detection limit: approx. 4.5 mg/dL

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean (mg/dL)	20	58	94
S.D. mg/dL	1.3	1.7	3.0
C.V. %	6.2	2.9	3.2

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean (mg/dL)	21	64	99
S.D. mg/dL	1.5	3.6	4.5
C.V. %	7.3	5.6	4.6

Analytical Sensitivity: approx. 0.027 abs for 10 mg/dL at 620 nm

Interferences (manual procedure):

Turbidity	Positive interference from 0.100 OD
Total bilirubin	Positive interference from 110 µmol/L
Ascorbic acid	No interference up to 2500 mg/dL
Glucose	No interference up to 1060 mg/dL
Haemoglobin	Positive interference from 10 µmol/L

Other substances may interfere (see § Limits)

Comparison studies with commercially available reagent:
Realised on spectrophotometer with specimens (n=95) between 10 and 130 mg/dL

y = 1.1008 + 0.01 r = 0,9941

Performances and stability data on Kenza 450TX/ISE and Kenza One are available on request.

CALIBRATION (8)

- Standard (vial R3) traceable to SRM 927.

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.
Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations

QUALITY CONTROL

- REF 95012 Urinary Control
- External quality control program

It is recommended to control in the following cases:
- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:
1. Prepare a fresh control serum and repeat the test
2. If control is still out of range, use a new vial of fresh calibrator
3. If control is still out of range, use a new vial of reagent and reassay

If control is still out of range, please contact BIOLABO technical support or your local Agent.

PROCEDURE

Refer to validated application of the Kenza Analyzer used

CALCULATION

The analyzer provides directly final result.
Refer to the instruction of use of Kenza analyzer.

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 512-530.*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p.916-919.*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-498 to 3-500 and 3-506 to 3-511*
(4) *Watanabe N. and al, Clin. chem. 32/8 (1986), 1551-1554.*
(5) *Le Bricon T., Ann. Biol. Clin. (2001),59, p.701-715*
(6) *Andries J. Bakker, Baukje Jellema, Ann. Biol. Chem., 36 (1999), p.163-1*
(7) *Fujita Y. and Al., Color reaction between pyrogallol red molybdenum complex and Protein. Bunseki Kagaku, 1983, 32, E379-E386*
(8) *SRM: Standard Reference Material ®*

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with